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(71) Applicant *(for all designated States except US)*: DUKE UNIVERSITY [US/US]; Erwin Road, Durham, NC 27706 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : WEISS, Jay, M. [US/US]; 26 South Hampton Place, Durham, NC 27705 (US). SUNDAR, Syam, K. [US/US]; 1801 Williamsburg Road, #35-C, Durham, NC 27707 (US).

(74) Agents: SIBLEY, Kenneth, D. et al.; Bell, Seltzer, Park & Gibson, P.O. Drawer 34009, Charlotte, NC 28234 (US).

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(54) Title: METHOD OF COMBATTING HIV INFECTIONS

(57) Abstract

Methods of combatting the effects of HIV infections are disclosed. The methods involve inhibiting the pituitary-adrenal axis, such as by administering an Interleukin-1 antagonist or a corticotrophin releasing factor antogonist as an active compound in an amount effective to combat the HIV infection. Among other things, the methods are useful for prolonging the latent period of HIV infection by administering the active compound to the subject during the latent period of HIV infection in an amount effective to prolong the latent period.

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METHOD OF COMBATTING HIV INFECTIONS

This invention was made with Government support under Grant Number MH45675 from the U.S. Public Health Service. The Government may have certain rights to this invention.

Field of the Invention

The present invention relates to methods of combatting HIV infections in general, and particularly relates to methods of combatting HIV infections by inhibiting the pituitary-adrenal axis.

Background of the Invention

The etiological agent in Acquired Immune Deficiency Syndrome (AIDS) has been identified as Human Immunodeficiency Virus (HIV). Once a person is infected with HIV, the virus establishes a latent infection in T helper cells. Infection of T helper cells is enabled by an envelope protein of HIV known as GP-120, which initially binds to the CD-4 receptor of T helper cells.

The latent period of HIV infection generally lasts several years, during which the infected individual may appear healthy. However, when the virus is activated, a productive infection is established. By this productive infection, more infectious virus is

produced while destroying the target T helper cells. Furthermore, conversion of a latent HIV infection to a productive (or "lytic") infection requires cell activation signals which can be elicited by agents that stimulate cell division or viruses such CMV, EBV, etc. CMV is one of the commonly observed opportunistic infections in AIDS, and it is noteworthy that latent CMV infections are activated in immunocompromised individuals. Therefore, any cofactor that can initiate transient immunosuppression may facilitate activation of latent viral (CMV) or opportunistic infections. When the host immune system mounts an immune response to these infections, the target T helper cells provide necessary signals for the conversion of latent HIV infection to a productive infection.

In addition to effects on T cells, HIV also results in degredation of cells within the brain and marked neurological changes leading to behavioral and psychological disturbance (i.e., AIDS dementia). Indeed, neurological disorders in HIV-infected individuals can occur even in the absence of the immunological abnormalities typically seen in AIDS subjects, and may be the only symptoms of HIV infection at a given time. *See, e.g.*, J. Berger, *Acta Neurol. Scand.* 77 (Suppl. 116), 40-76 (1988). HIV infection in brain precedes the development of neurological signs and symptoms. *See, e.g.*, P. Gallo et al., *AIDS Res. Hum. Retroviruses* 4, 211-221 (1988).

In the periphery, Interleukin-1 (IL-1) promotes immune responses. C. Dinarello, *Rev. Infect. Dis.* 6, 51-95 (1984). In the brain, on the other hand, IL-1 suppresses immune responses (S. Sundar et al., *Proc. Natl. Acad. Sci. USA* 86, 6398-6402 (1989)). This supression occurs through activation by IL-1 of the pituitary-adrenal axis and sympathetic nervous system through release of corticotrophin releasing factor (CRF). S. Sundar et al., *J. Neurosci.* 10, 3701-3706 (1990).

HIV enters the brain soon after infection, bringing with it integral protein constitutents such as GP-120. Regarding the influence of GP-120 on IL-1 in the body, L. Wahl et al., *Proc. Natl. Acad. Sci. USA* **87**, 621-625 (1989) suggest that GP-120 induces IL-1 and tumor necrosis factor (TNF) alpha in monocytes *in vitro*. No role for GP-120 located in the brain in the development of AIDS has heretofore been known.

Summary of the Invention

It has now been found that GP-120, when introduced into brain, induces IL-1 production in brain *in vivo*. Since IL-1 in the brain supresses immune responses, this finding suggests some important new mechanisms by which HIV infections evolve into AIDS.

In view of the foregoing, a method of combating HIV infections in a subject infected with HIV comprises administering to the subject (preferably during the latent period of HIV infection) an Interleukin-1 (IL-1) inhibitor or a corticotrophin releasing factor inhibitor (hereinafter referred to as the "active compound"). The active compound is administered in an amount effective to combat the HIV infection (e.g. to prolong the latent period of the infection).

Another aspect of the present invention is a method of delaying the onset of AIDS dementia in a subject infected with HIV, comprising administering to the subject (preferably during the latent period of HIV infection) an active compound as described herein, in an amount effective to delay the onset of AIDS dementia.

The active compounds described herein may be administered alone or in combination with other compounds useful for the therapeutic treatment of HIV infections, such as antiviral nucleosides.

Another aspect of the present invention is the use of the active compounds disclosed herein for the preparation of a medicament for combatting HIV

infections, prolonging the latent period of HIV infections, and delaying the onset of AIDS dementia.

Another aspect of the present invention is a composition comprising the active compounds disclosed herein and an antiviral nucleoside, together in an amount effective to prolong the latent period of an HIV infection in a pharmaceutically acceptable carrier.

While the applicants do not wish to be bound to any particular theory of operation of the instant invention, it should be noted that IL-1 very potently stimulates the pituitary-adrenal system via CRF to elevate plasma steroids. Elevated steroids are neurotoxic when acting in concert with other neurodegenerative or neurotoxic agents. *See, e.g.,* Sapolsky et al., *Brain Res.* **453**, 267-371 (1988). Preventing elevation of steroids via suppression of IL-1 should protect brain tissue from degeneration.

Brief Description of the Drawings

Figures 1A-C illustrate the effects of GP120 infusion into brain on plasma corticosterone concentration and natural killer (NK) cell activity. These Figures show that GP120 infusion elevates plasma corticosterone and depresses NK cell activity.

Figures 2A-E illustrate the effects of GP120 infusion into brain on IL-1 levels in brain, as determined with the thymocyte stimulation assay. These Figures show that IL-1 activity is detected in brain after GP120 is infused.

Figures 3A and **B** illustrate the effect of concurrent infusion of α-melanocyte stimulating hormone (α-MSH) with GP120 into brain on plasma steroid levels and NK cell activity. Since α-MSH blocks the effects of IL-1, these Figures indicate that the consequences of GP120 in brain (i.e., elevated steroids and depressed NK activity) are due to stimulation of IL-1 by GP120.

Figure 4 shows thymocyte stimulation (counts ^{3}H thymidine) of the Sephadex column fraction that showed

the highest activity in the Examples (fraction no. 12), and activity of the same fraction incubated with antibody to IL-1 receptor (MAb). Brain tissue was removed for fractionation 2.5 hrs after infusion of 4 μg GP120 into rat ventricle (rat 1,2), injection of 0.5 μg GP120 into rat hippocampus (rat 3,4), or injection of 0.5 μg GP120 into hippocampus of LPS-resistant C3H/HeH mice (M1, M2). The baseline for each animal is higest value observed in first six fractions or PHA-alone. "*" = response reaches criterion for IL-1 activity; i.e., 2x baseline.

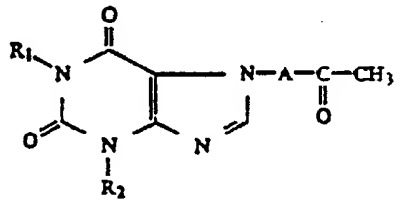
Detailed Description of the Invention

The method of the present invention may be employed to combat HIV infections or prolong the latent period of an HIV infection. HIV infections, when no longer latent, induce AIDS, which in turn induces a profound and marked state of constitutional disorder (*i.e.*, an appearance of general ill health and malnutrition) known as cachexia. By administering the active compounds disclosed herein during the latent period of HIV infection, the onset of AIDS, and in turn the onset of cachexia secondary to AIDS, are delayed. The present invention also provides a means for delaying the onset of AIDS dementia, which (as noted above) may preceed the immunosupression normally seen in AIDS.

Active compounds of the present invention may be administered by any suitable means, including both orally and parenterally (e.g., by intraperitoneal, intramuscular, and intraveneous injection).

Any Interleukin-1 inhibitor, including antagonists or agents which block the effects of IL-1, may be employed to carry out the present invention.

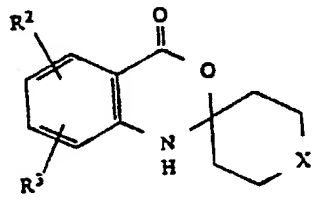
One group of IL-1 inhibitors are compounds according to Formula (I) below:



(I)

wherein R_1 and R_2 are the same or different and are selected from the group consisting of straight chain or branched chain alkyl radicals with 2 to 6 carbon atoms, cyclohexyl, straight chain or branched chain alkoxyalkyl and hydroxyalkyl radicals, and A represents a hydrocarbon radical with up to 4 carbon atoms which can be substituted by a methyl group. These compounds are known. *See* U.S. Patent No. 4, 965,271 to G. Mandell et al. (Applicant's specifically intend that the disclosures of all U.S. Patent references cited herein be incorporated herein by reference).

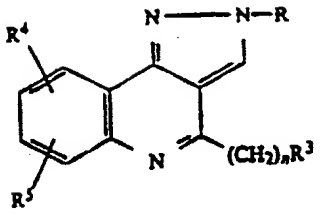
Another group of IL-1 inhibitors useful in practicing the present invention are compounds according to formula (II) below:



(II)

wherein X is CHR, NR, S or O; R is hydrogen, lower alkyl, lower alkenyl, or unsubstituted or substituted phenyl, naphthyl, pyridyl, quinolinyl, pyrazinyl, pyrimidinyl, quinoxalinyl or quinazolinyl, wherein the substituents are selected from halo, carboxy, lower alkoxycarbonyl, lower alkylsulfonyl, cyano, nitro, and trifluoromethyl; and R^2 and R^3 are each, independently, hydrogen, halo, lower alkyl, lower alkenyl, lower alkoxy, hydroxy, amino, mono-or diloweralkylamino, carboxy, lower alkoxycarbonyl, nitro or cyano. These compounds are known. *See* U.S. Patent No. 4,894,374 to J. Skotnicki and A. Abdel-Magid.

Still another group of IL-1 inhibitors are compounds according to Formula (III) below:



(III)

wherein R is phenyl, naphthyl, pyrid(-2-, -3- or -4-)yl, quinolin(-2-,-3-, or -4-)yl, or phenyl, naphthyl, pyrid(-2-.-3- or -4-)yl or quinolin(-2-, -3- or -4-)yl substituted by halo, lower alkyl, lower alkoxy, nitro, cyano, amino, monolower alkyl amino, dilower alkyl amino, carboxy, lower alkoxycarbonyl or hydroxy; R^1 is phenyl, phenyl lower alkyl, naphthyl, pyrid(-2-, -3- or -4-)yl, quinolin(-2-,-3-, or -4-)yl, pyrazin(-2- or -3-)yl, pyrimidin(-2-, -4- or -5-)yl, pyridazin(-3, -4- or -5-)yl, quinoxalin(-2- or -3-)yl or quinazolin(-2- or -4-)yl or any of the foregoing substituted with halo lower alkyl, carboxy, cyano, nitro, lower alkylsulfonyl, lower alkoxy carbonyl or lower alkyl substituted by fluoro, carboxy, cyano, nitro or lower alkoxy carbonyl; R^2 is hydrogen, lower alkyl, phenyl or benzyl; R^3 is NR^2R^1 or NR^6R^1; R^4 and R^5 are each independently, hydrogen, halo, lower alkoxy, lower alkyl, trifluoromethyl, cyano, nitro, carboxy or lower alkoxycarbonyl; R^6 is carbamoyl, phenylcarbamoyl, or halophenylcarbamoyl; and n is 1-5. These compounds are known. *See* U.S. Patent No. 4,748,246 to J. Skotnicki et al.

Other IL-1 antagonists include the following: (Z)-5-chloro-2,3-dihydro-3-(hydroxy-2 thienylmethylene)-2-oxo-1H-indole-1-carboxamide ("Tenidap™") and analogs thereof described in U.S. Patent No. 4,556,672 and incorporated herein by reference; [6-(4-fluorophenyl)-2,3-dihydro-5-(4-puyrinidyl)imidazo(2,1-b)thiazole] ("SK & F 86002") and analogs thereof; and (10-methoxy-4H-benzo[4,5]cyclohepta-[1,2-b]-thiophene-4-yliden) acteic acid ("IX 207-887") and analogs thereof, available from Sandoz, Basle, Switzerland.

The term lower alkyl as used herein refers to C1-6 alkyl and more preferably C1-3 alkyl such as methyl, ethyl and hexyl; the term lower alkoxy as used herein refers to C1-6 and more preferably C1-3 alkoxy such as methoxy, ethoxy, and hexoxy; the term halo as used herein refers to fluoro, chloro, or bromo.

Another group of IL-1 antagonists are those proteins known as Interleukin-1 receptor antagonist proteins. IL-1 receptor antagonist protein may be from any mammalian source (e.g., ovine, bovine, human) but is preferably human; IL-1 receptor antagonist protein may be used in glycosylated or unglycosylated form. Numerous examples of these proteins which may be used in practicing the present invention are known. An IL-1 receptor antagonist with a molecular mass (M_r) of about 22,000 (22K) is obtained from culture supernatants of adherent human mononuclear cells stimulated with immobilized immune complexes. *See, e.g.,* W. Arend et al., *J. Immunol.* **134**, 3868 (1985). An IL-1 receptor antagonist protein with a M_r of 18-25K is found in urine. *See, e.g.,* P. Seckinger, *J. Immunol.* **139**, 1546 (1987). An IL-1 receptor antagonist protein with a M_r of about 23K is released from cultured human monocytes which have been stimulated with granulocyte macrophage colony-stimulating factor (GM-CSF). *See* P. Roux-Lombard, *Cytokine* **1**, 45-51 (1989). An IL-1 Receptor antagonist protein with a M_r of about 25K obtained from human U937 myelomonocytic cells treated with phorbol myristate acetate and stimulated with GM-CSF has been cloned and produced by recombinant means in *Escherichia coli*. *See* D. Carter et al., *Nature* **344**, 633 (1990). A purified recombinant nonglycosylated 17K M_r produced in *E. coli* is described in W. Arend et al., *J. Clin. Invest.* **85**, 1694 (1990).

Still other IL-1 antagonists useful in practicing the present invention are disclosed in PCT Application WO 8911540 to Hannum et al. and PCT Application WO 8901946 to Dayer et al. Alpha-melanocyte

stimulating hormone (α-MSH) is currently preferred. It is understood that the term "alpha-melanocyte stimulating hormone" also includes any active fragment of this compound.

The actions of IL-1 in brain require mediation by CRF. S. Sundar et al., *J. Neuroscience* **10**, 3701-3706 (1990). Thus, blockade of CRF prevents suppression of cellular immune responses and elevation of steroids by IL-1 in brain. Consequently, CRF inhibitors may also be employed to carry out the present invention. Any CRF antagonist can be employed in practicing the present invention. For example, J. Rivier et al., *Science* **224**, 889-891 (25 May 1984), discloses the following CRF antagonists: ovine CRF residues 8 to 41, 9 to 41, and 10 to 41; α-helical CRF residues 8 to 41, 9 to 41, and 10 to 41; [Leu^{12}, Glu^{13}] α-Helical CRF residues 9-41; and [$Nle^{18,21}$] α-Helical CRF residues 8-41 and 10-41. Human CRF has been characterized as a 41-amino acid peptide having high homology with ovine CRF and the formula H-Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Ala-Arg-Ala-Glu-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Met-Glu-Ile-Ile-NH_2. One group of CRF antagonists based on this structure has the formula: Y-R_9-R_{10}-R_{11}-R_{12}-R_{13}-leu-leu-Arg-R_{17}-R_{18}-R_{19}-R_{20}-R_{21}-R_{22}-R_{23}-R_{24}-R_{26}-R_{27}-R_{28}-R_{29}-Gln-Ala-R_{32}-R_{33}-Asn-Arg-R_{36}-R_{37}-Nle-R_{39}-R_{40}-R_{41}-NH_2, wherein Y is an acyl group having 7 or less carbon atoms or hydrogen; R_9 is Asp or desR_9; R_{10} is Leu or desR_{10}; R_{11} is Thr, Ser or desR_{11}, R_{12} is (Q)D-Phe, D-Tyr, D-Leu, D-His, D-Nal, D-Pal, D-Ile, D-Nle, D-Val, D-Met, Phe or Leu; Q is H, 4Cl or 4F; R_{13} is His, Tyr or Glu; R_{17} is Glu, Asn or Lys; R_{18} is Val, Nle or Met; R_{19} and R_{24} are selected from the group consisting of Leu, Ile, ala, Gly, Val, Nle, Phe, Asn and Gln; R_{20} is Glu or D-Glu; R_{21} is Met, Nva, Ile, ala, Leu, Nle, Val, Phe or Gln; R_{22} is ala, Thr, Asp or Glu; R_{23} is Arg, Orn, Har or Lys; R_{25} is Asp or Glu; R_{26} is Gln, Asn or Lys; R_{27} is leu, Ile, ala, Val, Nva, Met, Nle, Phe, Asp, Asn, Gln or Glu; R_{28} is

Ala, Arg or Lys; R_{29} is Gln or Glu, R_{32} is His, Gly, Tyr or ala; R_{33} is Ser, Asn, leu, Thr or ala; R_{36} is Lys, Orn, Arg, Har or leu; R_{37} is leu or Tyr; R_{39} is Glu or Asp; R_{40} is Ile, Thr, Glu, ala, Val, leu, Nle, Phe, Nva, Gly or Gln; and R_{41} is ala, Ile, Gly, Val, leu, Nle, Phe, Nva or Gln; or a nontoxic addition salt thereof. In the foregoing, where the amino acid residue has isomeric forms, it is in the L-form of the amino acid that is represented unless otherwise expressly indicated. In addition, the following abbreviations are used herein: leu is either L-leucine or $C\alpha CH_3$-L-leucine (CML); ala is either L-alanine or $C\alpha CH_3$-L-alanine (CMA); D-Nal is D-alanine, the β-carbon of which is substituted with naphthalene and linked to the 1- or 2-carbon thereon; and D-Pal is D-alanine, the β-carbon of which is linked to the 3-position carbon of pyridine. These antagonists are known. *See* J. Rivier and W. Vale, PCT Application No. WO9003392 (1990). A preferred group of the foregoing antagonists are those having the formula: $Y\text{-}R_{12}\text{-}R_{13}$-leu-leu-Arg-$R_{17}\text{-}R_{18}\text{-}R_{19}\text{-}R_{20}\text{-}R_{21}\text{-}R_{22}\text{-}R_{23}\text{-}R_{24}\text{-}R_{25}\text{-}R_{26}\text{-}R_{27}\text{-}R_{28}\text{-}R_{29}$-Gln-ala-$R_{32}\text{-}R_{33}$-Asn-Arg-$R_{36}\text{-}R_{37}$-Nle-$R_{39}\text{-}R_{40}\text{-}R_{41}\text{-}NH_2$ wherein Y is Ac or hydrogen; R_{12} is D-Phe, D-Tyr, D-Leu, D-His, D-Nal, D-Pal, D-Nle, D-Ile, D-Val, D-Met or Phe; R_{13} is His, Tyr or Glu; R_{17} is Glu, Asn or Lys; R_{18} is Val, His, Tyr or Glu; R_{19} and R_{24} are selected from the group consisting of leu, Ile, ala, Gly, Val, Nle, Phe and Gln; R_{20} is Glu or D-Glu; R_{21} is Met, Nva, Ile, ala, leu, Nle, Val, Phe or Gln; R_{22} is ala, Thr, Asp or Glu; R_{23} is Arg, Orn, Har or Lys; R_{25} is Asp or Glu; R_{26} is Gln, Asn or Lys; R_{27} is leu, Ile, ala, Val, Nva, Met, Nle, Phe, Asp, Asn, Gln or Glu; R_{28} is ala, Arg or Lys; R_{29} is Gln or Glu; R_{32} is His, Gly, Tyr or ala; R_{33} is Ser, Asn, leu, Thr or ala; R_{36} is Lys, Orn, Arg, Har or leu; R_{37} is leu or Tyr; R_{39} is Glu or Asp; R_{40} is Ile, Thr, Glu, ala, Val, leu, Nle, Phe, Nva, Gly or Gln;and R_{41} is ala, Ile, Gly, Val, leu, Nle, Phe, Nva or Gln; and the pharmaceutically acceptable salts thereof. A particularly preferred subgroup of this group of

antagonists includes those in which: R_{12} is D-Phe, Phe or D-2Nal, R_{13} is His, R_{17} is Glu, R_{18} is Val, R_{19} and R_{37} are Leu, R_{20} is Glu or D-Glu, R_{21} is Nle, R_{22} is Ala, R_{23} is Arg, R_{24} and R_{28} are Ala, R_{25} and R_{39} are Glu, R_{26} is Gln, R_{27} is Leu, R_{29} is Gln, R_{32} is His, R_{33} is Ser, R_{36} is Arg, Lys, Har or Leu, R_{40} is Ile and R_{41} is Ala or Ile; and the pharmaceutically acceptable salts thereof. Specific examples of the foregoing include the following: [D-Phe^{12}, Nle^{21,38}]-human CRF(12-41); [D-Phe^{12}, Nle^{21,38}, Arg^{36}]-hCRF(12-41); [Nle^{21,38}]-hCRF(12-41); [Nle^{21,38}]-hCRF(9-41); [Nle^{38}]-Carp Urotensin I(12-41); [Nle^{21,38}, Arg^{36}]-hCRF(9-41); [D-2Nal^{12}, Nle^{21,38}]-hCRF(12-41); [D-Phe^{12}, Nle^{21,38}, Leu^{36}]-hCRF(12-41); [Acetyl-Asp^{9}, Gly^{19}, Nle^{38}, Asp^{39}, Nva^{40}]-hCRF(9-41); [Gln^{19}, Lys^{23}, Val^{24}, CMA^{33}, Nle^{38}]-hCRF(12-41); [Nle^{21,38}, Gly^{24}, Tyr^{32}, Orn^{36}]-hCRF(10-41); [Ala^{21}, Nle^{37}, Gln^{40}]-sauvagine(10-40); [Ala^{20}, Har^{22}, Nle^{37}, Phe^{39}]-sauvagine(11-40); [Val^{18,20}, Ile^{26}, Nle^{37}, Gly^{40}]-sauvagine(11-40); [4FD-Phe^{12}, CML^{14,15,19,27,33,37}, CMA^{22,32,41}, Nle^{38}]-AHC(12-41); [4ClD-Phe^{12}, Nle^{18,21,38}]-AHC(9-41); [D-Phe^{12}, Met^{27}, Nle^{21,38}]-AHC(12-41); [Nle^{18,38}, Leu^{21}, Ala^{27}]-AHC(12-41); [Leu^{12}, Glu^{13,22}, Lys^{26}, Nle^{38}]-AHC(12-41); D-Ile^{12}, Tyr^{13}, CMA^{28}, Nle^{38}]-AHC(12-41); [D-Leu^{12}, Glu^{13}, Ala^{33}, Nle^{38}]-AHC(12-41); [CML^{14,19,27,36}, Nle^{38}]-AHC(12-41); [D-Nle^{12}, Nle^{18,21,38}, Asn^{19}, Asp^{22}, Phe^{27}]-AHC(12-41); [D-Val^{12}, Nle^{21,38}, Ile^{24,27}, Nva^{41}]-hCRF(11-41); [Acrylyl-Leu^{10}, Val^{27}, Nle^{38}, Ala^{40}, Leu^{41}]-hCRF(10-41); [D-Tyr^{12}, Ala^{19}, Lys^{23}, Nle^{24,38,40} Nva^{27},]-hCRF(12-41); [D-His^{12}, CMA^{19,21,24,27}, Tyr^{32}, Thr^{33}, Nle^{38}, Gln^{40}]-hCRF(11-41); [4Cl-D-Phe^{12}, Nle^{19,27,38}, D-Glu^{20}, Nva^{21}, Leu^{24}, Gly^{40}, CMA^{41}]-hCRF(12-41); [Benzoyl-D-Met^{12}, CML^{21,24}, Har^{36}, Nle^{38}, Leu^{40}, Phe^{41}]-hCRF(12-41); [D-His^{12}, Phe^{21,24}, Orn^{23}, Asp^{27}, Nle^{38}, CMA^{40}, Val^{41}]-hCRF(12-41); [formyl-D-Pal^{12}, Phe^{19}, Gln^{21}, Thr^{22}, Tyr^{32}, Nle^{38}, CML^{40,41}]-hCRF(12-41); [D-Phe^{12}, Nle^{21,38}]-oCRF(12-41); [D-Phe^{12}, Nle^{21,38}, Arg^{36}]-oCRF(12-41). These compounds are known. *See* J. Rivier and W. Vale, *supra* (1990).

Additional CRF antagonists are peptides having the formula: Y-R_8-R_9-leu-R_{11}-R_{12}-R_{13}-leu-leu-Arg-R_{17}-R_{18}-R_{19}-Glu-R_{21}-R_{22}-R_{23}-R_{24}-R_{25}-R_{26}-R_{27}-R_{28}-R_{29}-Gln-ala-R_{32}-R_{33}-Asn-Arg-R_{36}-R_{37}-R_{38}-R_{39}-R_{40}-R_{41}-NH_2 wherein Y is acetyl, formyl, acrylyl, benzoly or hydrogen; R_8 is ala, Gly, Gln, Ile, leu, Nle, Phe, Val or des-R_8; R_9 is Asp, Glu or des-R_9; R_{12}, R_{19}, and R_{24} are selected from the group consisting of leu, Ile, ala, Asn, Gly, Val, Nle, Phe and Gln; R_{11} is Thr or Ser; R_{13} is His, Tyr or Glu; R_{17} is Glu, Asn or Lys; R_{18} is Val, Nle or Met; R_{21} is met, Nva, Ile, ala, leu, Gly, Nle, Val, Phe or Gln; R_{22} is ala or Thr or Glu; R_{23} is Arg, Orn, Har or Lys; R_{25} is Asp or Glu; R_{26} is Gln, Asn or Lys; R_{27} is leu, Ile, ala, Val, Nva, Met, Nle, Phe, Asp, Asn, Gin or Glu; R_{28} is ala, Arg or Lys; R_{29} is Gln or Glu, R_{32} is His, Gly, Tyr or ala; R_{33} is Ser, Asn, leu, Thr or ala; R_{36} is Lys, Orn, Arg, Har or leu; R_{37} is leu or Tyr; R_{38} is met or leu; R_{39} is Glu or Asp; R_{40} is Ile, Thr, Glu, ala, Val, leu, Nle, Phe, Nva, Gly or Fln; and R_{41} is ala, Ile, Gly, Val, leu, Nle, Phe, Nva or Gln; and the pharmaceutically acceptable salts thereof, as disclosed in U.S. Patent No. 4,605,642 to C. Rivier et al., the disclosure of which applicants specifically intend to incorporate herein by reference.

Dosage will vary depending on age, weight, and condition of the subject. Treatment may be initiated with small dosages less than optimum dose and increased until the optimum effect under the circumstances is reached. In general, the active compounds are preferably administered at a concentration that will afford effective results without causing any unduly harmful or deleterious side effects, and can be administered either as a single unit dose, or if desired, in convenient subunits administered at suitable times throughout the day. For example, the dosage of CRF antagonists as given is typically from about 0.01 to about 10 milligrams of the peptide per kilogram of the body weight of the subject.

Exemplary antiviral nucleosides which may be employed in conjunction with the present invention include 2',3'-dideoxyinosine; 3'-deoxythymidine; 3'-fluoro-3'-deoxythymidine; 2',3'-dideoxycytidine; 2',3'-dideoxy-5-fluoro-cytidine; 2',3'-dideoxyadenosine; 3'-azido-2',3'-dideoxyadenosine; 2'-fluoro-2',3'-dideoxyadenosine; 2',3'-dideoxy-2-fluoro-adenosine; 2',3'-dideoxy-2-chloro-adenosine; 2',3'-dideoxy-2-bromo-adenosine; 2',3'-dideoxy-2-amino-adenosine; 2',3'-dideoxyguanosine; 3'-azido-2',3'-dideoxyguanosine; 3'-azido-2',3'-dideoxyuridine; 2',3'-didehydro-2',3'-dideoxycytidine, and 2',3'-didehydro-2',3'-dideoxythymidine. *See* H. Mitsuya et al., *Proc. Natl. Acad. Sci. USA* **82**, 7096 (1985); H. Mitsuya and S. Broder, *Proc. Natl. Acad. Sci. USA* **83**, 1911 (1986); P. Herdewijn et al.; *J. Med. Chem.* **30**, 1276 (1987); C-H. Kim et al., *J. Med. Chem.* **30**, 862 (1987); V. Marquez et al., *Biol. Chem. Pharm.* **36**, 2719 (1987); T. Haertle et al., *J. Cellular Biochem.* Suppl. 11D, 65 (1987); J. Balzarini et al., *Biochem. Biophys. Res. Comm.* **145**, 277 (1987); M. Baba et al., *Biochem. Biophys. Res. Comm.* **145**, 1080 (1987); R. Schinazi et al., *J. Cellular Biochem.* Suppl. 11D, 74 (1987); Y. Hamamoto et al., *Antimicrob. Agents and Chemother.* **31**, 907 (1987). 3'-Azido-3'-deoxythymidine (or "AZT") is currently preferred.

The present invention may be employed to combat any type of human immunodeficiency virus, including HIV type 1 (HIV-1), HIV-2, and HIV-3, but is particularly useful for combatting HIV-1 infections.

The active compounds of the present invention (and, where included, antiviral nucleosides) may be administered to subjects per se or in the form of a pharmaceutically acceptable salt. When used in medicine, the salts of the active compounds should be both pharmacologically and pharmaceutically acceptable, but non-pharmaceutically acceptable salts may be used to prepare the free active compound or pharmaceutically

acceptable salts thereof and are not excluded from the scope of this invention. Such pharmacologically and pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, salicylic, p-toluenesulfonic, tartaric, citric, methanesulphonic, formic, malonic, succinic, naphthalene-2-sulphonic and benzenesulphonic. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group. Thus, the present invention also provides pharmaceutical formulations which comprise the active compound together with one or more pharmaceutically acceptable carriers thereof and optionally any other therapeutic ingredients. The carrier(s) must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not unduly deleterious to the recipient thereof.

The formulations of the present invention include those suitable for oral and parenteral (including subcutaneous, intramuscular and intravenous) administration.

The formulations may be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active compound into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active compound (and, where desired, an antiviral nucleoside) into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into desired formulations.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges,

each containing a predetermined amount of the potentiating agent as a powder or granules; or a suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion or a draught.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, with the active compound being in a free-flowing form such as a powder or granules which is optionally mixed with a binder, disintegrant, lubricant, inert diluent, surface active agent or dispersing agent. Molded tablets comprised of a mixture of the powdered active compound with a suitable carrier may be made by molding in a suitable machine.

A syrup may be made by adding the active compound to a concentrated aqueous solution of a sugar, for example sucrose to which may also be added any accessory ingredient(s). Such accessory ingredient(s) may include flavorings, suitable preservatives, an agent to retard crystallization of the sugar, and an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol (e.g., glycerol or sorbitol).

Formulations suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active compound, which is preferably isotonic with the blood of the recipient. Suitable aqueous carriers include sterile pyrogen-free water and sterile pyrogen-free isotonic saline solution.

The following Examples are provided to illustrate the present invention, and should not be construed as limiting thereof. In the Examples, μl means microliters, ml means milliliters, ng means nanograms, μg means micrograms, mM means milliMolar, M means Molar, cm means centimeter, G means gravity, μCi means microCuries, GBq means gigaBequerels, and temperatures are given in degrees Celsius unless otherwise indicated.

EXAMPLE 1

Animal Infusion Procedures

Male Sprague-Dawley rats weighing 200-300 grams were housed two per cage in enclosed, microisolator cages within laminar-flow racks. Animals were maintained on a twelve hour light (7:00 a.m.-7:00 p.m.) -dark (7:00 p.m.-7:00 a.m.) cycle.

Animals were infused through a cannula placed stereotaxically into various ventricles of the brain, such as a Lateral Ventrical (LV) or Fourth Ventricle, in accordance with known procedures. *See, e.g.*, J. Weiss et al., *Neuropharmacology* 25, 367-384 (1986). In some experiments, the cannula was cemented to the skull and the infusion administered to an awake animal 4-6 days later; in other experiments, anesthetized animals were infused immediately after the cannula was appropriately positioned into the brain. The substance infused was introduced slowly over ten minutes (for volumes 20 µl or less) or 15 minutes (40 µl). Except in Experiment 1 of Example 4 below, where effects at different times after infusion were studied, animals were sacrificed 2.5 hours after the infusion was completed.

EXAMPLE 2

Detection of IL-1 in Brain

Under halothane anesthesia, animals were perfused transcardially with 0.9% saline. For certain experiments, prior to infusion a blood sample was drawn by cardiac puncture or the spleen was removed after clamping the splenic artery. IL-1 was detected in brain by the procedure of A. Fontana et al. *J. Immunol.* 133, 1696 (1984). The brain was removed aseptically, meninges discarded, and brain stem plus diencephalon (a region found to show IL-1 activity in earlier studies), retained for analysis. Brain tissue was passed sequentially through sterile nylon wool meshes of 210 and 132 micrometer pore diameter to obtain dissociated cells rich in astroglia. Cells were sonicated (25 million cells/ml)

in ice cold RPMI medium containing protease inhibitors and centrifuged at 100,000 x G for 60 minutes. To remove low molecular weight substances (less than 10,000 daltons), the supernatant was subject to ultrafiltration using Amicon membranes (Amicon Corp., Danvers, MA).

After filtration through Amicon membranes, the supernatant was then subjected to Sephadex G-50 chromatography essentially as described by J. Cannon and C. Dinarello, *Science* **227**, 1247-1249 (1985). *See also* A. Fontana et al., *J. Immunol.* **133**, 1696 (1984); A Fontana et al., *J. Immunol.* **129**, 2413-2419 (1982). Sephadex G-50 (fine) was packed into 1 x 40 cm glass columns at a flow rate of 1 ml per minute, and the gel was equilibrated with eluting RPMI-1640 medium containing 25 mM HEPES buffer and 5 x 10^{-5} M 2-mercaptoethanol. Column preparation and fractionation was carried under sterile conditions in a laminar flow hood. Three hundred microliters of supernatant was loaded onto the columns and fractions (one per minute) were collected at a flow rate of 1 ml per minute. Columns were calibrated using calibration kit which consisted of substances of known molecular weights (Sigma Co.). All fractions were tested, in triplicate, for IL-1-like bioactivity in the thymocyte comitogenic assay as described below.

Thymus was collected from 4-6 week old endotoxin-resistant C3H/Hej strain of mice (Jackson Laboratories), and thymocytes were prepared by mincing the tissues between two sterile glass slides. The thymocytes were resuspended at a concentration of 10 million cells/ml in RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum and 1 μg/ml PHA. To each well of 96-well U-shaped-bottom microliter plates (Flow Laboratories) was added 100 μl of PHA-treated thymocytes and 100 μl of each fraction collected as described above. After incubation for 68 hours at 37° C, 1 microcurie of radioactive thymidine (NEN, Boston) was added to each well, and plates further incubated for four hours. The

cultures were then harvested onto glass fiber filters and incorporation of [^{3}H]thymidine determined in a liquid scintillation counter.

The criterion for positive IL-1 activity was that stimulation of thymocytes (^{3}H thymidine counts) in at least one of fractions 11-16 had to be twice as large as the highest of the initial six fractions (prior to void volume) and also had to be twice the PHA-control value. These 11-16 fractions were selected by the elution profile of recombinant human IL-1.

EXAMPLE 3

Immunological and Plasma Steroid Assays

After blood collection by cardiac puncture and removal of spleens under sterile conditions, mononuclear cells were separated on Ficoll/Hypaque density gradients. Viable lymphocytes were counted by trypan blue dye exclusion. Natural killer (NK) cell activity was determined by the method of Reynolds et al., *J. Immunol.* **127**, 282-287. To determine lymphocyte response to a mitogen, 0.1-ml aliquots of lymphocyte suspension [2 x 10^6 cells per ml in RPMI 1640 medium containing 10% (vol/vol) heat-inactivated fetal calf serum] were mixed with Conconavalin A at 10 μg/ml. After three days, 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine was determined. Plasma corticosterone was measured as described by Naylor et al. *J. Clin. Endocrinol. Met.* **67**, 404-406 (1988).

EXAMPLE 4

Effect of GP120 Infusion in Brain

These experiments were conducted to determine the effects of infusing (via indwelling lateral ventricle cannula as described above) different doses of GP120 and sacrificing animals at different intervals after the infusion. Three experiments (N = 12 each) were conducted. In the first, animals were infused with 4 μg GP120 (or 0.9% saline as control) and were sacrificed 2, 6, or 24 hours after infusion (at each time point, three animals were infused with IL-1 and one with saline). In

each of two subsequent experiments, animals were infused with 100 ng (N=4), 1.0 μg (N=4), or 0.9% saline (N=4) and sacrificed two hours post-infusion (second experiment) or six hours post-infusion (third experiment). Infusions were carried out so that all animals were sacrificed between 8 and 10 a.m.

Figures 1A-C show effects on plasma corticosterone concentration and NK cell activity. In **Figure 1A**, infusion of GP120 elevated plasma corticosterone, with the most consistent effect being seen at the earliest time point (two hours) after infusion. The effect decreased in longevity as the dose of GP120 given was reduced. NK cell activity was decreased in lymphocytes taken from both blood and spleen two hours post-infusion (see **Figure 1B**), and was decreased in blood lymphocytes at six hours (see **Figure 1C**). When mitogenic response to ConA was also measured (six hours post-infusion experiment), this was significantly reduced in blood lymphocytes of animals infused with 1.0 μg GP120 (data not shown).

Figure 2A demonstrates detection of IL-1 activity in the thymocyte assay following column fractionation. **Figures 2C-E** show an example of results from analysis of six animals whose brain tissue was fractionated on the same day and assayed together using the same pooled thymocytes; IL-1 activity was clearly evident in animals infused with 1 μg GP120. In this initial study, a total of 28 brains were analyzed (8 saline-infused, 20 GP120-infused); IL-1 was detected in 11 GP120-infused vs. 1 saline-infused (critical ratio: $Z = 1.97$, $p < .05$). With respect to IL-1 detection, neither a clear time-response nor dose-response was seen after intraventricular GP120; for animals sacrificed 2, 6, and 24 hours after infusion, number of animals IL-1 positive/number of animals assayed was 5/10, 4/7, and 2/3, respectively; for the doses 100 μg, 1 μg, and 4 μg, the values were 3/6, 3/5, and 5/9, respectively.

EXAMPLE 5

Blockade by Alpha Melanocyte Stimulating Hormone

Alpha-MSH blocks many of the biological actions of IL-1, including elevation of plasma steroid concentration and suppression of cellular immune responses produced by IL-1 in brain. To determine if elevated steroids and suppressed cellular immune responses following introduction of GP120 into brain resulted from elevated IL-1, α-MSH (10 ng) was infused together with GP120. Since α-MSH does not interfere with synthesis of IL-1, all animals in this study that were infused with GP120 were assayed for IL-1 in brain. The findings are shown in **Figures 3A** and **3B**. When animals were infused with GP120 (2 or 4 μg) via indwelling ventricular cannula and sacrificed 2.5 hours after infusion, 12 of 20 animals were positive for IL-1 in brain, a similar percentage as found in the initial study. Plasma steroid concentration was markedly elevated (see **Figure 3A**) and NK cell activity of blood lymphocytes reduced in animals infused with GP120 in which IL-1 activity was detected in brain (see **Figure 3B**). Infusion of α-MSH together with the GP120 blocked both the steroid elevation and reduced NK activity of animals whose brains were positive for IL-1. Infusion of α-MSH and 0.9% saline were without effect.

EXAMPLE 6

Potential Confounds: Indwelling Cannulation and LPS Contamination of GP120 Solution

Two potential confounding issues were addressed in overlapping experiments. First, it has been hypothesized that contact with blood induces expression of the CD4 antigen in brain microglia. Increased expression of the CD4 antigen in microglia was observed three days after disruption of blood-brain barrier, with peak antigen expression seen after five days. Consequently, implantation of an indwelling cannula, which would expose adjacent cells to blood, may have increased CD4 expression in these cells four to six days

later when GP120 was infused, thereby enabling GP120 to stimulate IL-1. To determine whether a surgical implantation four to six days prior to infusion enabled GP120 to stimulate IL-1, animals were infused with GP120 (i.c.v., 4 μg) immediately after the cannula was appropriately positioned, while animals were in the stereotaxic instrument. The animals were then sacrificed 2.5 hours later. In six animals, IL-1 activity was detected in the brains of five. Thus, inadvertent exposure of brain cells to blood several days before GP120 infusion to induce CD4 antigen expression does not account for stimulation of IL-1 in brain by GP120.

Contamination of LPS. Second, since LPS is a potent stimulator of IL-1, it was necessary to determine whether contaminating LPS in GP120 solution was responsible for IL-1 activity in brain. Although the GP120 used in these studies is subjected to sequential purification on immunoaffinity columns, and the final product has undetectable levels of LPS by limulus assay, additional experiments were conducted.

First, the ability of heat-inactivated (Δ) GP120 solution to induce IL-1 from macrophages in vitro was assessed. Initial studies indicated that heating (80-90°C for thirty minutes) rendered GP120 unable to stimulate IL-1 in brain. However, heating has been reported not to affect the ability of LPS to stimulate IL-1; therefore, possible presence of LPS could be assessed by determining whether ΔGP120 solution would stimulate IL-1. Macrophages were collected by peritoneal lavage of thioglycolate-injected rats and incubated in vitro with various concentrations of LPS (1.0, 0.1, .001, and .0001 μg/ml), the same concentrations of ΔLPS, and ΔGP120 solution (40 μl, the largest amount used in these studies, and 80 μl, 2x largest amount). After 48 hours, the presence of IL-1 in macrophage supernatant was tested for IL-1 in the thymocyte stimulation assay. Results are shown in Table 1.

TABLE 1: Thymocyte stimulation (net CPM ^{3}H thymidine incorporation) by supernatant of rat peritoneal macrophages incubated with various concentrations of LPS, heat-treated LPS, and various amounts of heat-treated GP120 solution.

Concentration (ug/ml)	LPS	Heat-treated LPS	Volume (ul)	Heat-treated GP120
1.0	30598	32436	80	2781
0.1	23649	26401	40	1935
0.001	12151	11330	20	1102
0.0001	4185	4938	10	422

CPM produced by supernatant of unstimulated macrophages = 3468; net CPM shown in table have this value subtracted. Values are the mean of triplicate determinations.

LPS potently activated IL-1 in macrophages in vitro in a dose-dependent manner. Equivalent thymocyte stimulation was produced by ΔLPS, thus confirming that heating of LPS did not diminish its ability to activate IL-1. Of most interest, the ΔGP120 solution was not devoid of activity in this assay, although the activity produced was quite low, being less than that produced by the lowest concentration of LPS used in this study. A regression analysis using the thymocyte stimulation produced by different concentrations of LPS indicated that the ΔGP120 solution produced stimulation equivalent to 1 pg/μl LPS.

Based on the foregoing, animals were infused with ΔGP120; this was done via chronic indwelling cannula as well as acutely. In addition, low doses of LPS equivalent to (or twice) the LPS concentration which the previous in vitro study indicated would account for the thymocyte stimulation of ΔGP120 solution was also infused. No IL-1 was seen in eleven animals following infusion of ΔGP120 acutely (N=6) or via chronic cannula (N=5), which compares with IL-1 detected in a total of 32 out of 52 brains from animals infused with GP120 (difference in percent of animals that were IL-1 positive when infused with GP120 vs. ΔGP120 is statistically significant [$p < .001$ by Fishers Exact Test]). Also, no IL-1 activity was detected in brains of any of six animals infused with low doses of LPS (four with 40 pg

[i.e., equivalent to 1 pg/μl in a 40 μl infusion] and two with 80 pg).

IL-1 stimulation by GP120 in brains of LPS-resistant C3H/HeJ mice. As a second test of whether stimulation of IL-1 in brain by GP120 might be caused by LPS, GP120 was infused into endotoxin-resistant C3H/HeJ mice. C3H/HeJ mice are resistant to effects of LPS derived from bacteria; therefore, any residual LPS in the GP120 solution would be less likely to stimulate IL-1 in these animals. Immediately after anesthesia with pentobarbital (25 mg/kg), C3H/HeJ mice were placed into the stereotaxic instrument and 0.5 μg (5 μl) of GP120 infused directly into the hippocampal region. Two and one-half hours later, animals were perfused and the brain tissue surrounding the infusion site (approximately 60 mg) analyzed for IL-1 activity. IL-1 activity was detected in five of six animals infused with GP120. IL-1 activity was not detected in any of six animals infused with ΔGP120. Also, no activity was detected in two animals infused with a large amount (500 pg) of LPS, confirming that this particular strain is resistant to LPS.

Direct injection of Gp120 into brain tissue. GP120 was injected directly (by acute procedure) into the dorsal hippocampus of five rats (coordinates [flat skull]: Posterior 5.0 mm [from bregma], lateral 2.5 mm, depth [from top of skull] 4.0 mm). The hippocampus was chosen based on studies showing a high concentration of IL-1 receptors in this brain region (29-31). For this procedure, a smaller amount of GP120 (0.5 μg [5.0 μl] infused over ten minutes) was given than was typically used for i.c.v. infusion. Two animals were similarly infused with an equal amount of ΔGP120. Two and one-half hours after completion of the infusion, each animal was perfused, and a unilateral (side of infusion) segment of dorsal hippocampus (50-60 mg) was analyzed for IL-1 activity. IL-1 activity was detected in all five animals

infused with GP120; no activity was detected in the hippocampal region of the two animals injected with ΔGP120.

Blocking of GP120-induced IL-1 activity by antibody to IL-1 receptor. To further determine that the activity observed in the thymocyte stimulation assay was due to IL-1, experiments examined whether a monoclonal antibody to mouse IL-1 receptor would block the thymocyte stimulation produced by cell lysate from GP120-infused brain. Monoclonal antibody specific to type 1 and 2 mouse IL-1 receptors (MAb) was used (Genzyme). At a concentration of 10 μg/ml, MAb blocked thymocyte stimulation in vitro produced by human recombinant IL-1β (^{3}H thymidine incorporation expressed as % of baseline radioactivity [i.e., PHA-alone]; 625 pg IL-1 = 2175% vs MAb + 625 pg IL-1 = 188%; 62.5 pg IL-1 = 493% vs MAb + 62.5 pg IL-1 = 99%). The addition of this antibody did not interfere with the assay; this was shown by testing responsivity of thymocytes to IL-2. Maximal thymocyte stimulation was achieved by 20 n of IL-2 (95 x 10^3% of PHA-alone) and this response was undiminished in the presence of 10 μg/ml of MAb (118 x 10^3% of PHA-alone). Six animals were then infused with GP-120; two rats with 4 μg i.c.v. (acute cannulation), and two rats and two C3H/HeJ LPS-resistant mice with 0.5 μg injected into the hippocampus. Brain tissue (brain stem + diencephalon for i.c.v. infused and approximately 60 mg of hippocampal region tissue for injection) was removed from perfused animals 2.5 hours later, after which brain cell lysate was fractionated and tested for IL-1 activity as described previously. The results, shown in **Figure 4**, revealed that IL-1 activity, present in five of the six animals, was blocked by antibody to IL-1 receptor, thereby indicating that thymocyte stimulation of GP120-infused brain was due to IL-1.

The foregoing examples are illustrative of the present invention, and are not to be construed as

limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. A method combatting HIV infections in a subject in need of such treatment, comprising administering to the subject an active compound selected from the group consisting of Interleukin-1 (IL-1) inhibitors and corticotrophin releasing factor inhibitors in an amount effective to prolong said latent period.

2. A method according to claim 1, wherein said active compound is an IL-1 inhibitor.

3. A method according to claim 1, wherein said active compound is α-melanocyte stimulating hormone or a pharmaceutically acceptable salt thereof.

4. A method according to claim 1, wherein said active compound is human monocyte Interleukin-1 receptor antagonist protein or a pharmaceutically acceptable salt thereof.

5. A method according to claim 1, wherein said active compound is a corticotrophin releasing factor inhibitor.

6. A method according to claim 5, wherein said corticotrophin releasing factor inhibitor is [D-Phe^{12}, $Nle^{21,38}$]-human CRF (12-41) or a pharmaceutically acceptable salt thereof.

7. A method according to claim 1, wherein said active compound is administered parenterally.

8. A method according to claim 1, wherein said active compound is administered orally.

9. A method of prolonging the latent period of HIV infections in a subject infected with HIV, comprising administering to the subject during the latent period of HIV infection an active compound selected from the group consisting of Interleukin-1 (IL-1) inhibitors and corticotrophin releasing factor inhibitors in an amount effective to prolong said latent period.

10. A method according to claim 9, wherein said active compound is an IL-1 inhibitor.

11. A method according to claim 9, wherein said active compound is α-melanocyte stimulating hormone or a pharmaceutically acceptable salt thereof.

12. A method according to claim 9, wherein said active compound is human monocyte Interleukin-1 receptor antagonist protein or a pharmaceutically acceptable salt thereof.

13. A method according to claim 9, wherein said active compound is a corticotrophin releasing factor inhibitor.

14. A method according to claim 13, wherein said corticotrophin releasing factor inhibitor is [D-Phe^{12}, $Nle^{21,38}$]-human CRF (12-41) or a pharmaceutically acceptable salt thereof.

15. A method according to claim 9, wherein said active compound is administered parenterally.

16. A method according to claim 9, wherein said active compound is administered orally.

17. A method of delaying the onset of AIDS dementia in a subject infected with HIV, comprising administering to the subject prior to the onset of AIDS dementia an active compound selected from the group consisting of Interleukin-1 (IL-1) inhibitors and corticotrophin releasing factor inhibitors in an amount effective to delay the onset of AIDS dementia.

18. A method according to claim 17, wherein said active compound is an IL-1 inhibitor.

19. A method according to claim 17, wherein said active compound is α-melanocyte stimulating hormone or a pharmaceutically acceptable salt thereof.

20. A method according to claim 17, wherein said active compound is human monocyte Interleukin-1 receptor antagonist protein or a pharmaceutically acceptable salt thereof.

21. A method according to claim 17, wherein said active compound is a corticotrophin releasing factor inhibitor.

22. A method according to claim 21, wherein said corticotrophin releasing factor inhibitor is [D-Phe^{12}, $Nle^{21,38}$]-human CRF (12-41) or a pharmaceutically acceptable salt thereof.

23. A method according to claim 17, wherein said active compound is administered parenterally.

24. A method according to claim 17, wherein said active compound is administered orally.

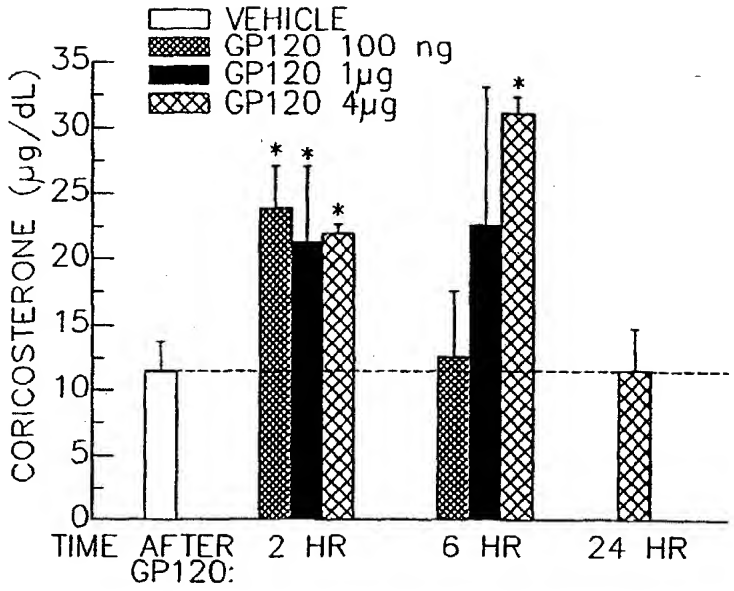


FIG. 1A.

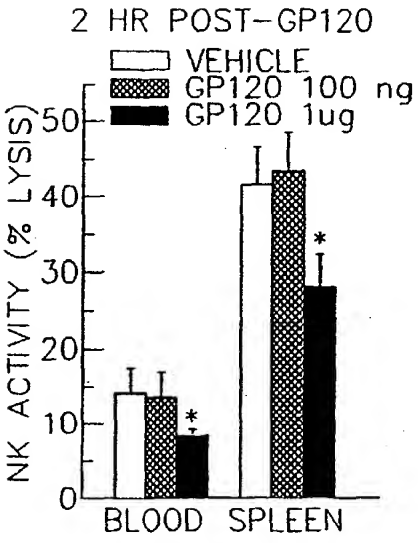


FIG. 1B.

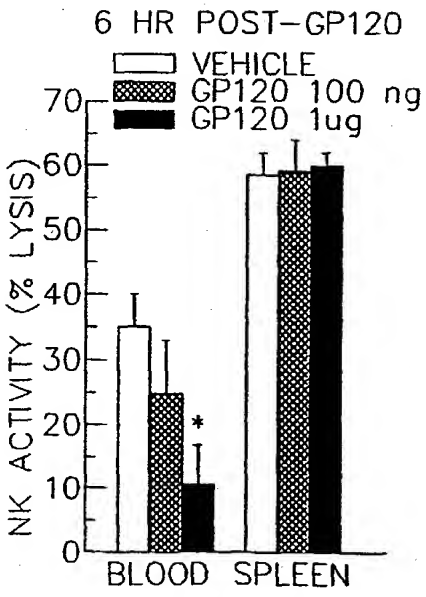
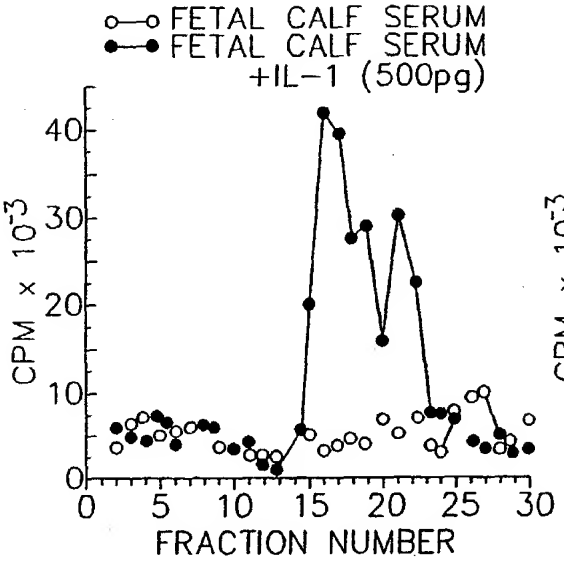


FIG. 1C.

FIG. 2A.



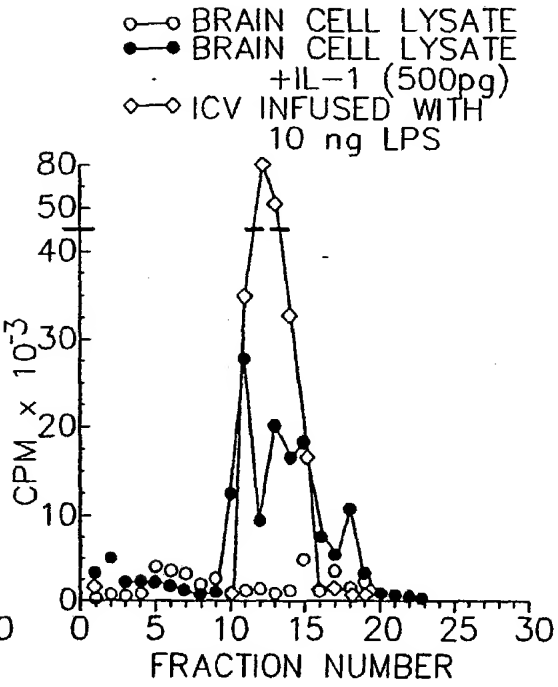


FIG. 2B.

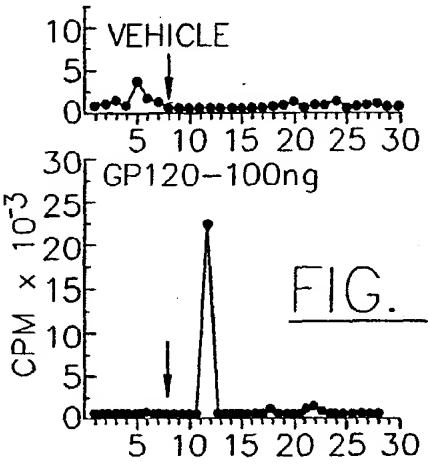


FIG. 2C.

FIG. 2E.

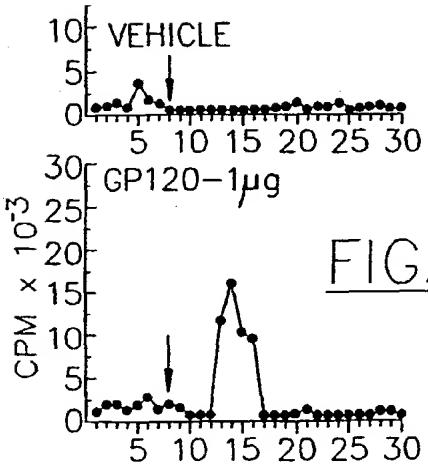
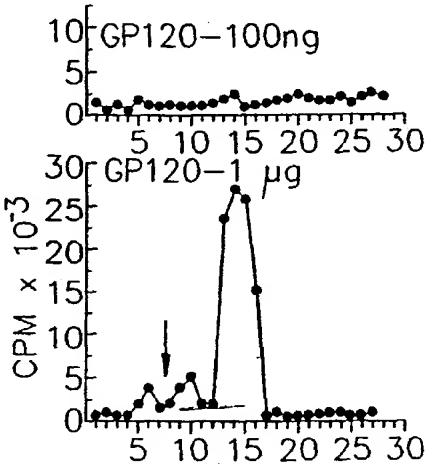


FIG. 2D.



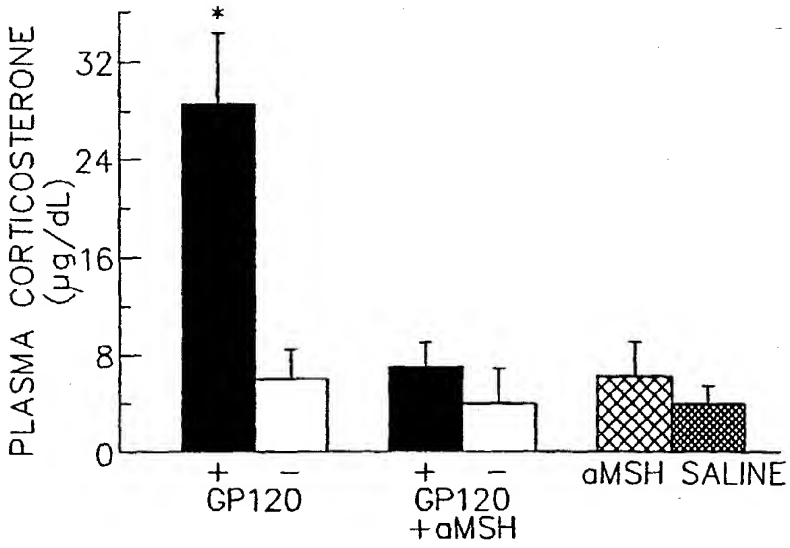

PLASMA CORTICOSTERONE (μg/dL)
32
24
16
8
0
*
+ GP120
− GP120
+ GP120 +αMSH
− GP120 +αMSH
αMSH SALINE

FIG. 3A.

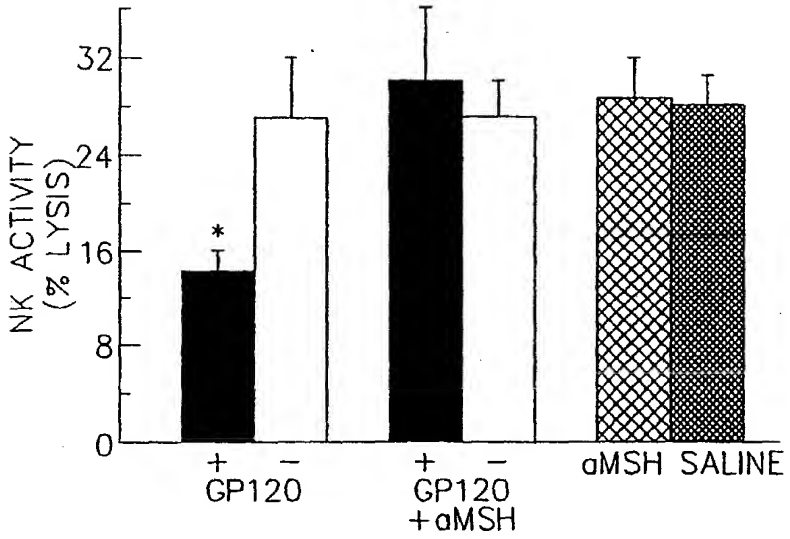

NK ACTIVITY (% LYSIS)
32
24
16
8
0
*
+ GP120
− GP120
+ GP120 +αMSH
− GP120 +αMSH
αMSH SALINE

FIG. 3B.

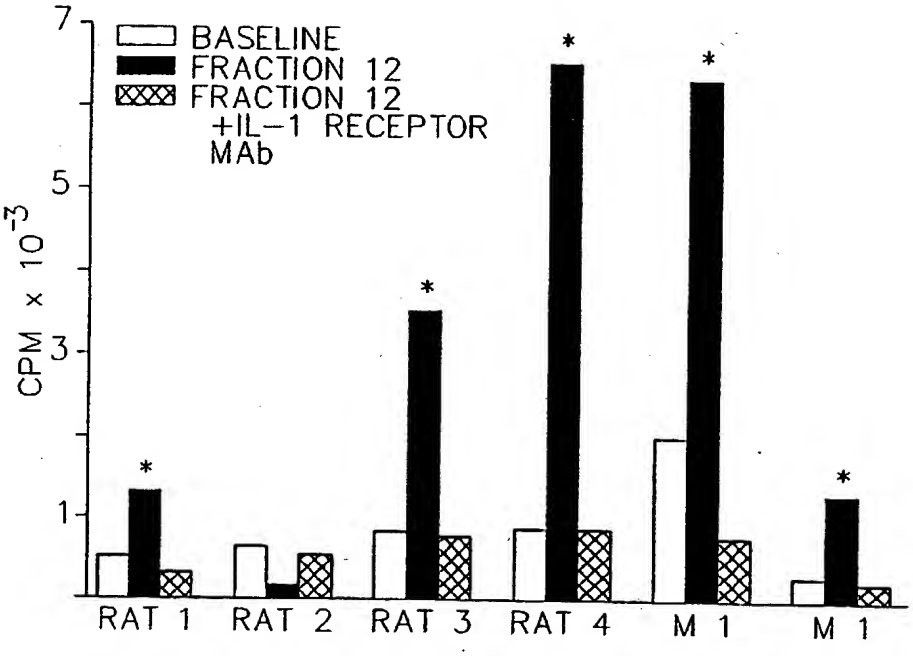

BASELINE
FRACTION 12
FRACTION 12
+IL-1 RECEPTOR
MAb
CPM x 10^{-3}
7
5
3
1
*
RAT 1
RAT 2
RAT 3
RAT 4
M 1
M 1

FIG. 4.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/06320

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02, 37/24
US CL :514/12, 21
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

dialog, medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP, A, 0,403,251 (Hanna) 19 December 1990, entire document.	1-24
Y	The Journal of Neuroscience, volume 10, No. 11, issued November 1990, Sundar et al, "Brain IL-1-Induced Immunosuppression Occurs Through Activation of Both Pituitary-Adrenal Axis and Sympathetic Nervous System by Corticotropin-releasing Factor," pages 3701-3706, entire document.	5-6, 13-14, 21-22
Y	Diabetologia, volume 34, issued 1991, Eizirik et al, "An interleukin-1 receptor antagonist protein protects insulin-producing Beta cells against suppressive effects of interleukin-1ß," pages 445-448, entire document.	1-2, 4, 7-10, 12, 15-18, 20, 23-24
Y	Proc. Natl. Acad. Sci, USA, volume 86, issued August 1989, Sundar et al, "Intracerebroventricular infusion of interleukin 1 rapidly decreases peripheral cellular immune responses," pages 6398-6402, entire document.	1-3, 7-11, 15-19, 23-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 18 September 1992	Date of mailing of the international search report 28 SEP 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer CHOON P. KOH Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Inter...ional application No.
PCT/US92/06320

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: _
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any addtional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention frist mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.